

Desacetylcephalosporin C

FIG. 1

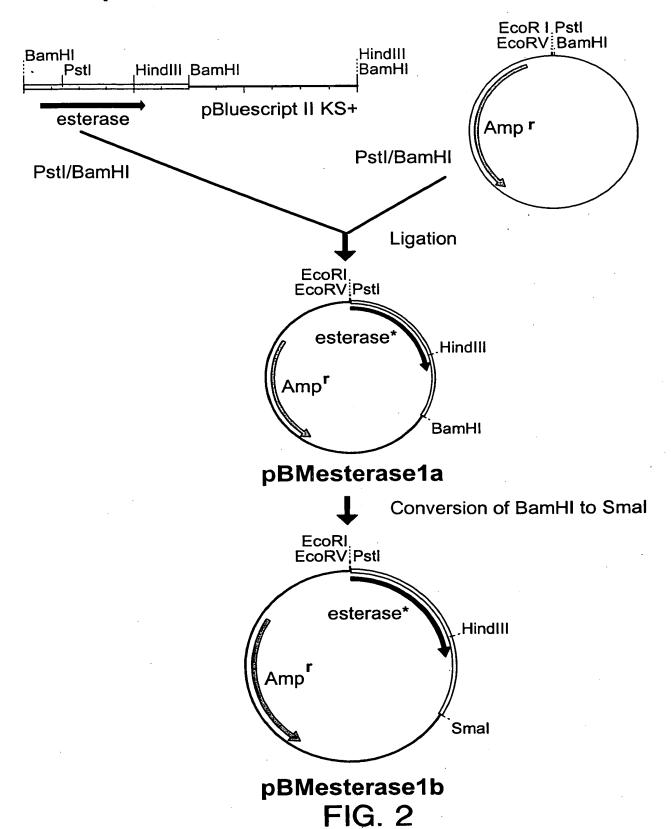
Compound X

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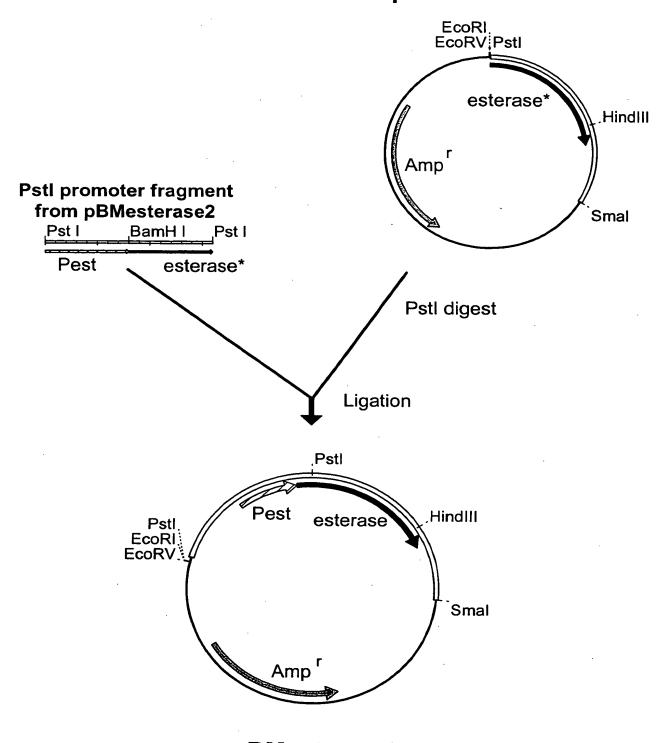
pBMesterase1

pBluescript II KS+



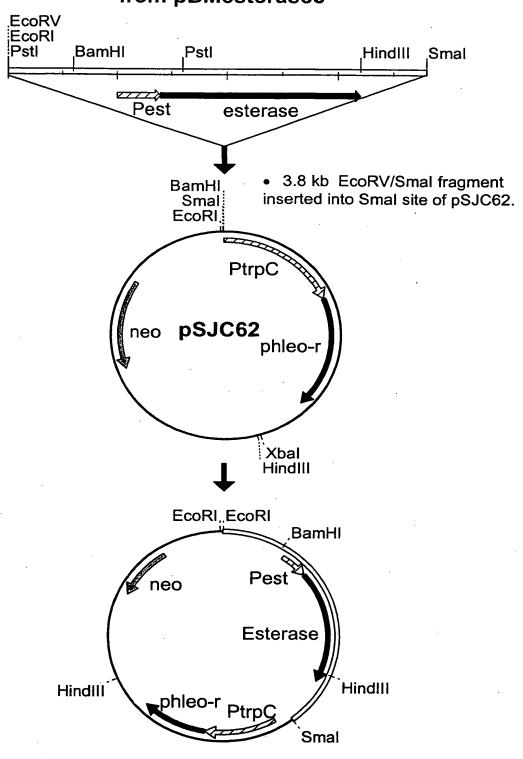
O.G. FIG.

pBMesterase1b



pBMesterase3 FIG. 3

Smal/ EcoRV esterase fragment from pBMesterase3



pSJC62.3 FIG. 4

. 49 FW FW

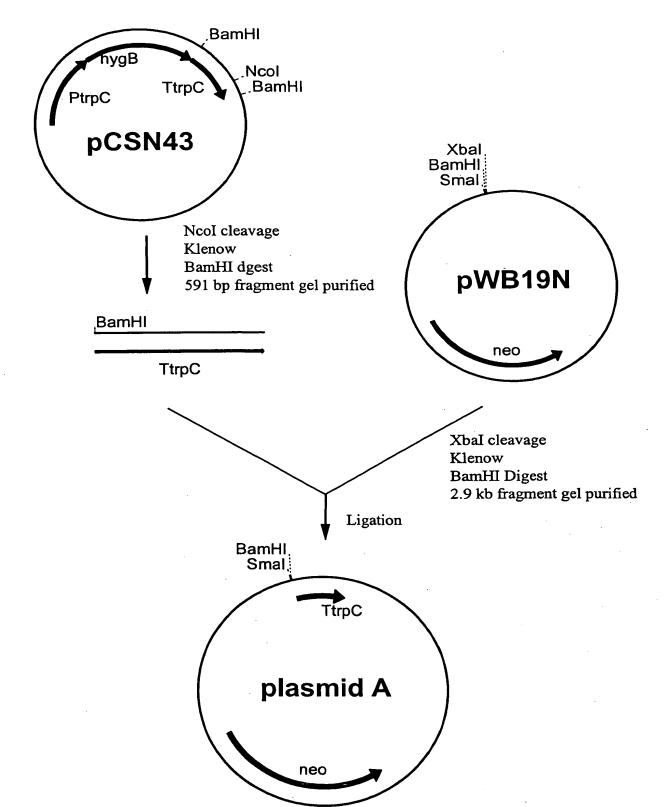


FIG. 5

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O

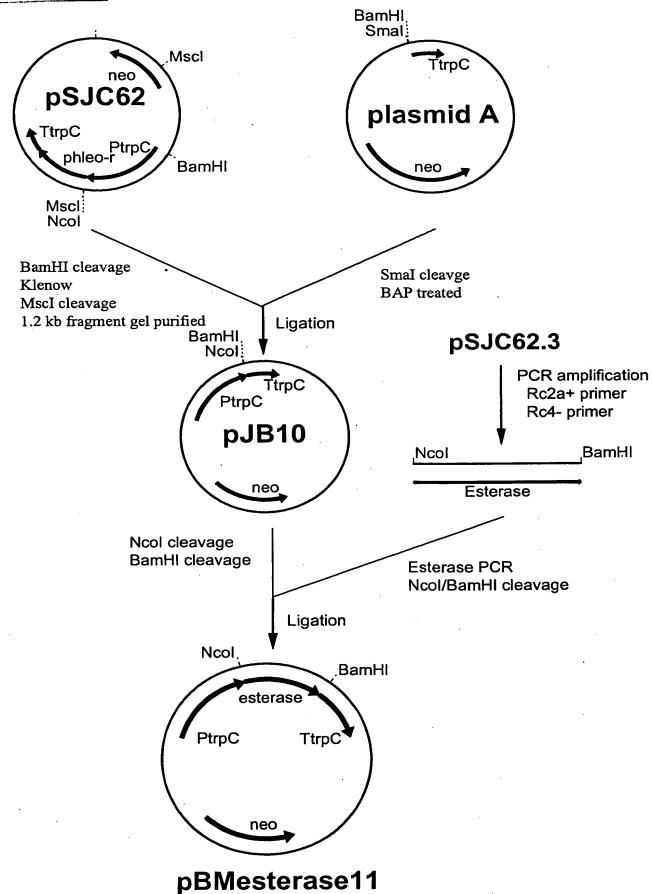


FIG. 6

N-TERMINAL AMINO ACID SEQUENCE

TNPNEP AMINO ACID SEQ. ACX AAPy CCX AAPy GAPu CC **REV. TRANSLATION GGPy TCPu TTX GGPu TTX INVERSE** GGPy TCPu TTG GGPu TTX GT **PROBE** 2 3

Four 17-mer oligonucleotide probes each with a 32-fold degeneracy were synthesized from the N-terminal amino acid sequence and used to probe a Southern blot of R.toruloides DNA.

FIG. 7

RHODOSPORIDIUM ESTERASE CDNA

TA	GCTC	CT	CAA1	CTC	CTTC	CACC	CTC	CGC	CTC	CCT	CGC'	TGC	GAC	GCT	CCA	GCT	CGC	CTT'	TGCC	. 70
M	L	L	N	L	F	T	L	A	s	Ļ	A	A	T	L	Q	L	A	F	A	. •
TC'	rcco	SAC	CTC	CCT	CGT	CCG	CCG	CAC	GAA	CCC.	AAA	CGA	GCC	ccc	TCC	CGT	CGT	CGA	CCTC	130
S	P	T	S	L	V	R	R	T	N	P	N	E	P	P	P	V	V	Ď	L	
GG	CTA	CGC	CCG	CTA	CCA	AGG	CTA	CTT	GAA	CGA	GAC	CGC	CGG	ACT	CTA	CTG	GTG	GCG	CGGA	190
G	Y	A	R	Y	Q	G	Y	L	N	E	T	A	G	L	Y	W	W	R		
ΑT	CCG	CTA	CGC	CTC	GGC	TCA	GCG	CTT	CCA	GGC	TCC	TCA	GAC	GCC	CGC	GAC	GCA	CAA	GGCC	250
Ι	R	Y	A	S	A	Q	R	F	Q	A	P	Q	T	P	A	T	H	K	A	
GT	CCG	CAA	CGC	GAC	TGA	GTA	TGG.	ACC	GAT	CTG	TTG	GCC	GGC	TAG	CGA	.GGG	AAC	CAA	CACG	310
V	R	N	A	T	E	Y	G	P	I	С	W	P	A	s	E	G	T	N	T	
AC	CAA	GGG	CTT	GCC	GCC	GCC	TAG	CAA	CAG	CTC	GAG	CAG	CGC	GCC	:GCA	GAA	ACA	GGC	GTCG	370
T	K	G	L	P	P	P	S	N	S	s	S	s	A	P	Q	K	Q	A	S	
GA	GGA	TTG	CCT	CTT	CCT	CAA	TGT	CGI	TGC	ccc	CGC	CGG	CTC	GTG	CGA	GGG	CGA	CAA	TCTT	430
	D																			
CC	CGT	CCI	CGT	CTA	CAT	TCA	CGG	AGG	TG	CTA	ACGC	CTI	CGG	CGA	ATGO	GAG	CAC	CGG	CAGC	490
P	V	L	V	Y	I	H	G	G	G	Y	A	F	G	D	A	S	T	G	S	
G	CTT	TGC	CGC	CTI	CAC	CAA	GCA	CAC	GGG	SAAC	CAF	AGAI	rggr	CGI	TGT	'AA	TCI	CCA	GTAC	550
D	F	A	A	F	T	K	H	T	G	T	K	M	V	V	V	N	L	Q	Y	
C	STCI	CGC	CAC	CTI	TG	TTI	CCI	CGC	CTG	GCC	AAGO	CAI	rga,	AGG	ACTA	ACGO	TGI	'AAC	GAAC	610
R	L	G	S	F	G	F	L	A	G	Q	A	M	ĸ	D	Y	G	V	T	N	
G	CCGG	CT	rgcz	TG	ACC	AGC	TA	CG	ccc:	rtc?	TAA	GGT	TC	AAC	AGC2	ACG?	CTC	GAA	GTTC	670
A	G	L	L	D	Q	Q	F	A	L	Q	W	V	Q	Q	H	V	S	K	F	
G	GCGG	CA	ACCO	CCGI	ATC!	ACGI	CTAC	CGA:	TTT	GGG	GCG	AGT	CTG	CAG	GCG	CAGO	GTC	CCGI	TATG	730
G	G	N	P	D	H	٧	T	I	W	G	E	S	A	G	A	G	S	V	М	
A	ACC	\GA:	rca?	rtg	CGA	ACGO	GCGC	CA	ACA	CCG:	rca)	AGG	CTC	rcg	GTC:	rca <i>i</i>	AGA!	AGCO	CCTC	790
N	Q	I	I	A	N	G	G	N	T	V	K	A	L	G	L	K	K	P	L	
T'	rcc <i>i</i>	ACG	CTG	CA	rcg	GCT	CTC	CCG'	TCT'	TCC'	rcc	CCT	ACC	AAG	CCA	AGT	ACA	ACTO	cccc	850
F	Н	A	A	I	G	S	S	V	F	L	P	Y	Q	A	K	Y.	N	S	P	
T'	TCG	CCG	AGC:	rgc:	CT	ACT	CCC	AAC'	TCGʻ	TCT	CGG	CGA	CAA	ACT	GCA	CCA	AAG	CGG	CTCG	910
F	A	Ε	L	L	Y	S	Q	L	V	s	A	T	N	С	T	K	A	A	s	• • •
T	CCTI	rcg	CTT	GCC:	rcg/	AAG	CTG	rcg	ACG	CTG	CGG	CGC	rcg	CTG	CGG	CGGG	GCG'	rgaz	AGAAC	970
S	F	A	С	L	E	A	V	D	A	A	A	L	A	A	A	G	V	K	N	2.0
T	CGG	CGG	CGT'	rcc	CGT'	rcg	GGT'	rtt	GGT	CGT	ATG'	TCC	בפהי	יים:	ፐርር፡	ארפי	GG 2	-CTr	יכשיים	1030
	Δ																			1000

FIG. 8A

AC:	rga.	GCG	CGC	GTC	GCT	CCT	TCT	CGĊ	CAA	GGG	CAA	GAA	CAA	ССТ	ממח	TGG	ממכ	CCT	CTTC	1090
T	E	R	A	s	L	L	L	A	K	G	K	K	N	L	N	G	N	L		1090
AC	CGG	GAT	CAA	CAA	ССТ	CGA	CCN	N C C	שים ע	~ n m	3 mm	~~							CGAC	
т	G	T	N	N	~ T	CON	COV	700.		CAT.	ATT	CAC	TGA	CGC	CAC			GAA	CGAC	1150
•	Ŭ	-	14	14	ı	υ	£	G	F.	1	F	Т	D	A	T	I	Q	N	D	
AC	GAT	CAG	CGA	CCA	GTC	GCA	GCG	CGT	СТС	CCA	בייים	CCA	ccc	CCT	CCT			CCM	CTTC	
T	I	S	D	Q	S	Q	R	v	s	Q	F	D	R	L	L	A	G	L	F'	1210
CC	СТА	САТ	CAC	ירייר.	CCA	GGN	CCC	CC 3		~~m										
Þ	v	Т	T		GG7	ADD.	aca	CCA	<u> </u>	CGT	CGC	GAA	GCA	GTA	CCC	GAT	CTC		CGCG	1270
•	•	-	1	s	E	E	R	Q	A	V	A	K	Q	Y	P	I	S	D	A	
CC	GTC	AAA	GGG	CAA	CAC	СТТ	СТС	TCG	תב ח	СТС	ccc	CCT	~ n m	~~~		CMC			CGTC	
P	s	K	G	N	T	F	٠,٠	D	T	CIC	7 2		CAI	- GC	.GGA	CTC	.GAC		CGTC	1330
	_		Ŭ	••	•	-	3	K	_	3	A	٧	T	A	D	S	T	F	V	
TG	ccc	GAC	CTA	CTG	GAC	CGC	CGA	.GGC	GTT	CGG	СТС	יהייר	רכר	CCA	ממח.	ccc	CCT	C T T	CGAC	1200
С	P	T	Y	W	T	Α	E	A		٦٥٥	~	S		H			L			1390
																_	_	-	D	
TACGCGCCGGCTCACCACGCGACAACTCGTACTACATCGGCTCCATCTGGAACGGC														1450						
Y	A	P	A	H	H	A	T	D	N	S	Y	Y	I	G	s		W			1420
7 T	~ n n	CMC														,				
nn T	GAA	GTC	.GG1	CTC	GTC	CGT	CCA	GTC	CTT	CGA	CGG	CGC	GCT	CGG	CGG	CTI	CAT	CGA	GACG	1510
r	K	S	V	s	S	V	Q	S	F	D	G	A	L	G	G	F	I	E	T	·
TT	CAA	CCC	GAZ	ACAA	CAA	ACGC	ידכר	ממט.	ממר.	GNC	~ » m	~~~	~~~						CGAC	
F	N	P	N	N	N	Δ Δ	אר	N	t t	.GAC	CWI	CAA		TTA	CTC	GCC	GAC			1570
-	•	•	••	.,	14	A	A	Į,	Α.	Т	1	N	P	Y	W	P	T	F	D	•
TC	GGG	CAA	\GC#	AGCI	CCI	CTT	CAA	CAC	GAC	GAC	CAC	:CC»	CAC	ССТ	ירייר	ישיכר		-C-3	CCCG	1.520
S	G	K	0	L	L	F	N	T	T	·Ozzo	D	תטטת	m m		CIC	.100	الالال		CCCG	1630
														L				_	Þ	
CG	CAI	CGI	TGF	AGAC	TTC	CAAC	CTI	'GAC	CGA	CTT	TGG	CAC	GAG	CCA	CDE	CAC	ממסי	стс	CGAC	1690
R	Ι	V	E	T	S	s	L	T	D	F	G	T	s	Q				C		1090
														_		_		-	_	
TT	CTG	-600	TGC	GTC	CAA	CTC	GGI	'GAA	CGC	GGG	TCT	'C								1726
r.	W	R	G	S	I	S	V	N	A	G	L									•

FIG. 8B

GGATCCACCCGAACTCTGTCCCGCTTTCTGGCTTTCTTCCTTGCTGTCGCCCCATCGCCT 60 |-- Translation Start --> 120 MLLNLFTLASLAATL - Mature peptide -> CCAGCTCGCCTTTGCCTCTCCGACCTCCTCGTCCGCCGCACGAACCCAAACGAGCCCCC 180 QLAFASPTSLVRRTNPNEPP TCCCGTCGTCGACCTCGGCTACGCCCGCTACCAAGGCTACTTGAACGAGACCGCCGGACT 240 PVVDLGYARYQGYLNETAGL CTACTGGTGGCGCGAATCCGCTACGCCTCGGCTCAGCGCTTCCAGGCTCCTCAGACGCC 300 YWWRGIRYASAQRFQAPQTP CGCGACGCACAAGGCCGTCCGCAACGCGACTGAGTATGGACCGATCTGTTGGCCGGCTAG 360 ATHKAVRNATEYGPICWPAS CGAGGGAACCAACACGACCAAGGGCTTGCCGCCGCCTAGCAACAGCTCGAGCAGCGCGCCC 420 EGTNTTKGLPPPSNSSSAP GCAGAAACAGGCGTCGGAGGATTGCCTCTTCCTCAATGTCGTTGCCCCCGCCGGCTCGTG 480 QKQASEDCLFLNVVAPAGSC CGAGGGCGACAATCTTCCCGTCCTCGTCTACATTCACGGAGGTGGCTACGCCTTCGGCGA 540 EGDNLPVLVYIHGGGYAFGD TGCGAGCACCGGCAGCGACTTTGCCGCCTTCACCAAGCACGCGGAACCAAGATGGTCGT 600 ASTGSDFAAFTKHTGTKMVV TGTAAATCTCCAGTACCGTCTCGGCAGCTTTGGTTTCCTCGCTGGCCAAGCCATGAAGGA 660 V N L Q Y R L G S F G F L A G Q A M K D [---- Intron #1 -----CTACGGTGTAACGAACGCCGGCTTGCTTGACCAGGTGAGTTTCCCGCATGATACCCGCCC 720 YGVTNAGLLDQ ACCTTTCGACTCATGCTGACGCCTCTCCCGCTCGCAGCAATTCGCCCTTCAATGGGTTCA 780 QFALQWVQ ACAGCACGTCTCGAAGTTCGGCGGCAACCCCGATCACGTTACGATTTGGGGCGAGTCTGC 840 Q H V S K F G G N P D H V T I W G E S A [---- Intron #2 -----AGGCGCAGGGTCCGTTATGAACCAGATCATTGCGAACGTGAGCCACCCGAACCGATCTCC 900 GAGSVMNQIIAN 960 GGNT CCGTCAAGGCTCTCGGTCTCAAGAAGCCCCTCTTCCACGCTGCCATCGGCTCCTCCGTCT V K A L G L K K P L F H A A I G S S V F TCCTCCCTACCAAGCCAAGTACAACTCCCCCTTCGCCGAGCTGCTCTACTCCCAACTCG 1080 LPYQAKYNSPFAELLYSQLV FIG. 9A

CTGC A	GGC A	GCT L	CGC A	TGC A	GG(A	G G	GCG [,]	TGA K	AGA 1	AAC 1	TC S	GGC A	GG(CGT F	TCC F	CG	TT F	CG(GGT F	TTT W	GG	S	1200
CGTA Y	TG1 V	rccc P	GGI V	Y V	CG D	ACG G	GGA T	CCT F	TCI I	rtc L	AC T	TGA E	GC(R	GCG A	CG7 S	rce	CT L	CC	rtc L	TCG A	CC	CA K	1260
AGGG G	CA# K	\GAJ K	AGAA N	ACC:	rca. N	ATG G	GCG	TGC	- : GT	Int	:ro :GA	n 1 GCI	3 TT	CGA	GTG	GC 1	rrc	AG	GAT	CTC	G	T	1320
GACA			SACO				GAA	CCI L													T	GA	1380
GTT	- Ir	otro GTC	on i	4 GGC	TCT	 GTT	cgc	CCA	GC	GAC	SAC	TG	 \CT	 TGT	TC	rr:	 rrc	SCG.	 Aag	 ATI	 (A)] CG	1440
ATT(I	ATT(F	CAC	rga D	CGC A	CAC T	TAT I	TCA Q	GA. N	AC(SAC O	AC(T	SAT I	CAG S	CG?	AC(2AG	STC S	GCA Q	GCC R	SC(GT V	1500
CTCC	CAC Q	GTT(F	CGA(CCG R	CCT L	CCT L	CGC A	CGC G	CC'	TC:	rtc F	P P	CTA Y	CAT I	CAC T	CC	rco S	GA E	GGA E	GCC R	GC(CA Q	1560
GGC(CGT V	CGC(GAA(K	GCA Q	GTA Y	CCC	GAT I	CTC S	CG.	ACC	GCG A	CC(STC S	AAA K	.GG(GC2	AAC V	AC T	CTT F	CTC S	T	CG R	1620
CATO			CGT V								GTG	TG(CGT	Int	roi CCC	n (GT(# 5 :G1	 CT	TCT	ccc	GA	 GT	1680
ATT	CCG	CTG	ACT	TCC	cgc	TTC	ecc	:GC#	\ĞC	TG(C	CCC P	GAC T	CCI Y	roa [.]	GGZ	AC(T	CGC A	CCG. E	AGG A	CG:	rt F	CG G	1740
GCT S	CGT S	CCG A	CCC. H	ACA K	AGG	GCC I	TCI	TCC	SAC	TA(Y	CGC A	GC(CGG A	CTC	AC(CA(H	CGC A	GA T	CCG	ACI	A.A V	CT S	1800
CGT: Y	ACT. Y	ACA I	TCG G	GCT S	CCA I	TCI	rgga 1 1	ACC	GC 3	AA(K	GA? K	GT S	CGG V	TCI	CG	TC(S	CGI V	CC Q	AGT S	CC:	rt F	CG D	1860
ACG G	GCG A	CGC L	TCG G	GCG G	GCI F	TCA	OTL I	AGA	r CG	TT F	CA.	P	CGA N	ACA	ACI	AA N	CGC A	TG A	CCA N	ACI	A.A K	GA T	1920
CCA	TCA N	ACC	CTT Y	ACT W	GGC I I	CG?	CG1	TCC	SAC O	TC S	GGC G	SCA. K	AGC	AGC	TC	CT(L	CTT F	CA N	ACA T	CG	AC r	GA T	1980
CGA R	GGG D	ACA T	CCC	TCI	CTC	CCC	CCC	SAC(CG P	CG R	CA?	7 V	TTC E	AGA	CT	rcz S	AAC S	CT L	TGA T	CCC	GA O	CT F	2040
TTG G	GCA T	CGA S	GCC Q	AGA K	AGA I	CCA	LAGI	GC(SAC	TT F	CTC W	GGC R	GTG	GG1	CA	AT(CTC S	GG' V	TGA N	ACC	GC A	GG G	2100
GTC [*]	TCT		CGT	CTI	TCC	TTC	CGZ	CT	rcc	TT	CG	rtc	TTI	CG1	TG	ľľ.	ra1	TC	TTG	CAC	ЭT	TC	2160
CGT	TGT	ATC	GGC	CAI	TCC	TGC	GTC					CGA			ACC	GT.	rgo	CA.	AGT	GC	GA	AA	2220

TRN 2-1738 RHODOSPORIDIUM ESTERASE CDNA\$

FIG. 10

The first start that other wast them to that could that that that the

Amino acid composition from 1 to 572 TRN 2-1738 RHODOSPORIDIUM ESTERASE CDNA

To	tal	Percent		•
A	67	11.7		
C	7	1.2		
D	25	4.4		
Ē	16	2.8		
F	35	6.1		
G	49	8.6		
H	9	1.6		
I	21	3.7		
K	25	4.4		
L	48	8.4		
M	4	0.7		
N	35	6.1		
P	31	5.4		
Q	26	4.5		
R	16	2.8		
S	52	9.1		
T V	43	7.5		
W	32	5.6		
Y	10	1.7		
ı	21	3.7	•	
Acidic	41	7 2		
Basic	41	7.2		
Charged	82	7.2 14.3		
Net charge	Õ	0.0		
Hydrophobic	136	23.8		
		23.0		
Residues	572			

MW 61334

FIG. 11